

sequence MF  $\alpha$  of *S. cerevisiae* that is a peptide comprised of 13 amino acid residues may be employed. The MF  $\alpha$  signal sequence has a protease site determined by the sequence of amino acids Lys-Arg-Glu-Ala (SEQ ID NO:26). During the cloning process in pPIC9 of a human insulin precursor gene, this gene may be preferably inserted into the site Xho I that eliminates the -Glu-Ala- residues, whereby the starting insert of the human insulin gene is maintained immediately after the proteases removal site (Figure 1). The cloning in site Xho I permits to obtain a precursor released into the culture medium without remaining amino acids belonging to the signal peptide, thus simplifying the steps of purification of the human insulin. --

Replace lines 3-19 at page 41 with the following text.

**--Primers:**

SEQ ID NO:1: 5'-TCACACCTGG TGGAAGCTCT CTACCTAGTG TGCAGGG -3'

SEQ ID NO:2: 5'-GGTCTTGGGT GTGTAGAAGA AGCCTCGTTC CCCGCACACT  
AGGTA-3'

SEQ ID NO:3: 5'- TTTGTGAACC AACACCTGTG CGGCTCACAC CTGGTGGAA -  
3'

SEQ ID NO:4: 5'-GCTGGTACAG CATTGTTCCA CAATGCCACG CTTGGTCTTG  
GGTGT -3'

SEQ ID NO:5: 5'-CTAGTTGCAG TAGTTCTCCA GCTGGTAGAG GGAGCAGATG  
CTGGTACAGC AT-3'

**Final Product:**

SEQ ID NO:6: 5'- TTTGTGAACC AACACCTGTG CGGCTCACAC CTGGTGGAAAG  
CTCTCTACCT AGTGTGCGGG GAACGAGGCT TCTTCTACAC ACCCAAGACC  
AAGCGTGGCA TTGTGGAACA ATGCTGTACC AGCATCTGCT CCCTCTACCA  
GCTGGAGAAC TACTGCAACT AG -3'

(complete insulin precursor) --

Replace lines 1-21 at page 42 with the following text.

--Construction of an insulin precursor by the polymerase chain reaction (PCR) with the codons more utilized by *Pichia pastoris*.

**Primers:**

SEQ ID NO:7: 5'-ACTTGGTTGA AGCTTGAC TTGGTTGTG GTGAAAGAGG  
TTTCTTCTAC-3'  
SEQ ID NO:8: 5'-AGAAGTACAA CATTGTTCAA CGATACCTCT CTTAGTCTT  
GGAGTGTAGA -3'  
SEQ ID NO:9: 5'-ACACTTGTGT GGTTCTCACT TGGTTGAAGC TTT-3'  
SEQ ID NO:10: 5'- TTACTCGAGT TAGTTACAGT AGTTTCCAA TTGGTACAAA  
GAACAGATAG AAGTACAACA TTGTTTC -3'  
SEQ ID NO:11: 5'-CCGCTCGAGA AGAGATTGT TAACCAACAC TTGTGT -3'

The obtained product contains the following sequence:

SEQ ID NO:12:  
5'-TTTGTAAACC AACACTTGTG TGGTTCTCAC TTGGTTGAAG CTTGTACTT  
GGTTTGTGGT GAAAGAGGTT TCTTCTACAC TCCAAAGACT AAGAGAGGTA  
TCGTTGAACA ATGTTGTACT TCTATCTGTT CTTGTACCA ATTGGAAAAC  
TACTGTAAC AA-3'--

Replace lines 12-20 at page 43 with the following text.

**--Example 3**

**Construction of Factor  $\alpha$  with preferences codons of *Pichia pastoris***

By means of this technique the nucleotide sequence corresponding to the leader sequence or signal peptide was cloned.

The employed primers were the following:

SEQ ID NO:13: 5'-CGCGGATCCA AACCATGAGA TTCCCATCTA TCTTCAGTC  
TGTTTGTTG GCTGCT -3'--

Replace lines 1-16, at page 44 with the following text.

--SEQ ID NO:14: 5'- GTTTGTTCG CTGCTTCTTC TGCTTGGCT GCTCCTGTTA  
ACACTACTAC TGAAGACGAA ACTGCTCA-3'

SEQ ID NO:15: 5'-ACGTCGAAGT CACCTTCAA GTCAGAGTAA  
CCGATAACCG CTTCAGCTGG GATTGAGCA GTTTCGTCTT C -3'

SEQ ID NO:16: 5'-GATGAACAAAC AAACCATTAT TAGTAGAGTT  
AGAGAAAGGC AAAACAGCAA CGTCGAAGTC ACCTTC -3'

SEQ ID NO:17: 5'-CCGCTCGAGA GAAACACCCCT CTTCCCTAGC AGCGATAGAA  
GCGATAGTAG TGTTGATGAA CAACAAACCA TT -3'

The final product has the following sequence:

SEQ ID NO:18

5'-ATGAGATTCC CATCTATCTT CACTGCTGTT TTGTTCGCTG CTTCTTCTGC  
TTGGCTGCT CCTGTTAACAA CTACTACTGA AGACGAAACT GCTCAAATCC  
CAGCTGAAGC GGTTATCGGT TACTCTGACT TGGAAAGGTGA CTTCGACGTT  
GCTGTTTGC CTTCTCTAA CTCTACTAAT AATGGTTGT TGTTCATCAA  
CACTACTATC GCTTCTATCG CTGCTAAGGA AGAGGGTGT TCTCTCGAGA  
AGAGAGAGGC TGAAGCA-3'--

Replace lines 7-23 at page 45 with the following text.

--3- The PCR product SEQ ID NO:18 was digested with the same restriction enzymes utilized in 1 and was ligated to the fragment obtained in 2.

4- The vector obtained in 3 and the PCR fragment SEQ ID NO:12 was digested with the XhoI, and subsequently they were ligated.

5- The recombinants having the correct orientation of the insulin precursor insert were detected by the HpaI.

#### EXAMPLE 4

##### **Cloning of insulin precursor gene in pPIC9 yeasts vector**

The DNA fragment encoding the insulin precursor was amplified by PCR, employing as a template SEQ ID NO:6 previously obtained and as primers the following sequences:

SEQ ID NO:19: 5' -GGGGATCCAT ATGCTCGAGA AAAGATTGTT  
GAACCAACAC CTGT-3'--

Replace lines 1-2 at page 46 with the following text.

--SEQ ID NO:20: 5' -TTAGAATTCC CGGGTCTAGT TGCAGTAGTT CT- 3'--

Replace lines 1-4 at page 49 with the following text.

--SEQ ID NO:19: 5' - GGGGATCCAT ATGCTCGAGA AAAGATTGTT  
GAACCAACAC CTGT-3'

SEQ ID NO:21: 5'-TCACTCGAGC GGTCTAGTTG CAGTAGTTCT-3'--

Replace lines 16-20 at page 58 with the following text.

--His probe that is a fragment 1587 bp of HIS4 gene obtained by digestion of vector Ppic9 with the MscI and the Ins probe that is a fragment 227 bp obtained by PCR employing as a

template the plasmid pPIC9IB and the primers corresponding to the sequences SEQ ID NOS:19 and 20.--

Replace lines 11-13 at page 62 with the following text.

--Sequence of primers:

5' AOX I: 5' - GACTGGTTCC AATTGACAAG C (SEQ ID NO:25)

3'AOX IN: 5' - GTCGTGGTTT CTCATAGTAG AGTGGAC (SEQ ID NO:22)--

Replace lines 24-25 at page 63 with the following text.

--Gap primers:

Gap5': 5' GGT CAT CAC TGC TCC AT (SEQ ID NO:23)--

Replace lines 1 at page 64 with the following text.

--Gap3': 5' AGC AGC ACC AGT GGA AGA (SEQ ID NO:24)--

Replace lines 17-20 at page 64 with the following text.

--Insulin precursor primers:

SEQ ID NO:19: 5'- GGGGATCCAT ATGCTCGAGA AAAGATTGT  
GAACCAACAC CTGT

SEQ ID NO:21: 5'- TCACTCGAGC GGTCTAGTTG CAGTAGTTCT--